REMARKS

Claims 124 and 139 have been amended. Claims 151-155, 157, 158, 160-164, 166, and 167 have been canceled. Claims 124-127, 129-150, 156, 157, 159, 165, and 168 are pending in the present application.

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the above amendments and the following remarks is requested.

I. Rejection of Claims 152, 154, 161, and 163 under 35 U.S.C. § 112, First Paragraph

According to the Advisory Opinion, Claims 152, 154, 161, and 163 would be rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. The Office Action states:

> The Examiner has been unable to locate adequate support for cyclohexadepsipeptide synthetase genes encoding polypeptides having 75% or 85% sequence identity to the polypeptide of SEQ ID NO: 2. Thus, there is no indication that methods/mutant cells that require the disruption or deletion of a genus of cyclohexadepsipeptide synthetase genes encoding polypeptides having 75% or 85% sequence identity to the polypeptide of SEQ ID NO: 2 were within the scope of the invention as conceived by Applicants at the time the invention was filed.

Claims 152, 154, 161, and 163 were cancelled rendering the rejections moot. For the foregoing reason, Applicant respectfully requests withdrawal of the rejections.

II. Rejection of Claims 124-128, 131-143, and 146-150 under 35 U.S.C. § 112, First Paragraph

According to the Advisory Opinion, Claims 124-128, 131-143, 146-150, 155, 157-158, 160-164, and 166-167 would be rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. The Office Action states:

> Proposed amended claims 124-127, 131-142, 146-150 would remain rejected and proposed new claims 155, 157-158, 160-164, 166-167 would be rejected under 35 USC 112, first paragraph, because the specification ... does not reasonably provide enablement for (a) a method for producing a secreted heterologous protein using a mutant Fusarium venenatum cell wherein said cell comprises a nucleic acid having a disruption or deletion in a Fusarium venenatum cyclohexadepsipeptide synthetase gene, wherein said gene encodes a polypeptide

having at least 70%, 75%, 80%, 85% or 90% sequence identity to the polypeptide of SEQ ID NO: 2, or wherein said gene hybridizes to the polynucleotide of SEQ ID NO: 1 under the medium or medium-high stringency conditions recited in the claims, or (b) a mutant Fusarium venenatum cell modified to produce less cyclohexadepsipeptide synthetase than the corresponding wild type Fusarium venenatum cell by disruption or deletion of the cyclohexadepsipeptide synthetase gene, wherein the cyclohexadepsipeptide synthetase gene encodes a polypeptide having at least 70%,75%,80%,85% or 90% sequence identity to the polypeptide of SEQ ID NO: 2, or wherein said gene hybridizes to the polynucleotide of SEQ ID NO: 1 under the medium or medium-high stringency conditions recited in the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

This rejection is respectfully traversed for reasons of record and reasons stated below.

Claims 155, 157, 158, 160-164, 166, and 167 have been canceled.

The Office alleges that the specification "fails to provide any teaching or suggestion as to which are the structural elements in the only cyclohexadepsipeptide synthetase gene (SEQ ID NO: 1) disclosed that can be modified such that one would obtain structural homologs of (a) the gene of SEQ ID NO: 1 which encode cyclohexadepsipeptide synthetases having at least 70%, 75%, 80%, or 90% sequence identity to the polypeptide of SEQ ID NO: 2, or (b) the gene of SEQ ID NO: 1 which hybridize under medium or medium-high conditions to the polynucleotide of SEQ ID NO: 1 and encode cyclohexadepsipeptide synthetases."

Applicant is simply disrupting or removing a portion of a cyclohexadepsipeptide synthetase gene sequence so expression of the gene is disrupted and no cyclohexadepsipeptide is produced. It is well known in the art that by selecting a conserved or homologous region of a known gene based on sequence comparisons to other similar genes known in the art, a deletion vector can be prepared without knowledge of the corresponding gene sequence in a cell and disrupt that corresponding gene. Moreover, the presence of a cyclohexadepsipeptide synthetase gene in a cell can be determined by employing the known gene, or a portion thereof, as a probe in Southern hybridization analysis under varying stringency conditions. Applicant has shown that the deduced amino acid sequence (SEQ ID NO: 2) of the cyclohexadepsipeptide synthetase gene of SEQ ID NO: 1 shares approximately 59% identity with the cyclohexadepsipeptide synthetase gene (*esyn1*) of *Fusarium scirpi* (Haese *et al.*, 1993, *Mol. Microbiol.* 7: 905-914; DNA sequence listed in EMBL database under accession number Z18755). This sequence comparison indicates there are regions of conserved homology between the sequences at the DNA level, which can be used to construct a disruption or deletion vector for use in another *Fusarium venenatum* cell

without any knowledge of the DNA sequence in that cell. This state of art is demonstrated by Herrmann *et al.* (*Molecular Plant-Microbe Interactions* 9: 226-232, 1996) who showed that an internal fragment of the *Fusarium scirpi* cyclohexadepsipeptide synthetase gene was useful in disrupting the *Fusarium avenaceum* cyclohexadepsipeptide synthetase gene without any knowledge of the full nucleic acid sequence of the *Fusarium avenaceum* gene.

With the information provided by Applicants in the specification and the knowledge available in the pertinent art, one skilled in the art can construct disruption or deletion vectors for transformation into any *Fusarium venenatum* cell, shown to produce cyclohexadepsipeptide, to disrupt or delete a cyclohexadepsipeptide synthetase gene without knowledge of the gene's sequence. For example, a DNA fragment containing a conserved or homologous region interrupted with a selectable marker or a DNA fragment with a portion of the conserved or homologous region removed by digestion with a restriction enzyme can be used with reasonable predictability to replace the corresponding similar gene via homologous recombination in a *Fusarium venenatum* cell that produces cyclohexadepsipeptide.

Applicants assert, therefore, that it is well within the skill of the art to make cyclohexadepsipeptide-deficient *Fusarium venenatum* cells using the nucleic acid sequences disclosed in the specification and the prior art without being provided with the corresponding DNA sequences encoding the enzymes involved in the biosynthesis of cyclohexadepsipeptide. The need for isolation of the gene of a *Fusarium venenatum* cell, delineation of the nucleic acid sequence, and a determination of which modifications would lead to deficient production of cyclohexadepsipeptide is not necessary to disrupt or delete a gene involved in the biosynthesis of cyclohexadepsipeptide.

However, to further prosecution, Applicants have amended claims 124 and 139 to recite in part: "wherein the cyclohexadepsipeptide synthetase gene encodes a cyclohexadepsipeptide synthetase having an amino acid sequence which has at least 95% identity with SEQ ID NO: 2; or a cyclohexadepsipeptide synthetase which is encoded by a nucleic acid which hybridizes under at least high stringency conditions with (i) the nucleic acid of SEQ ID NO: 1, (ii) the cDNA of SEQ ID NO: 1, or (iii) a complete complementary strand of (i) or (ii), wherein high stringency conditions are defined as prehybridization and hybridization at 45°C in 5X SSPE, 0.3% SDS, 200 μg/ml sheared and denatured salmon sperm DNA, and 50% formamide and washing three times each for 15 minutes using 2X SSC, 0.2% SDS at 55°C ..."

For the foregoing reasons, Applicant submits that the new claims overcome the rejections under 35 U.S.C. § 112, first paragraph. Applicant respectfully requests reconsideration and withdrawal of the rejection.

III. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

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